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from the hinder portion of this cells, are cut off, which wander in and join the "yolk cells," but have nothing to do with the formation of the mesenteron. The remainder of the primary entoderm differentiates into two large lateral and a middle solid longitudinal band, and in the former there appears in each segment a cavity. The outer wall of this cavity forms the somatopleure, the inner the splanchnopleure and epithelium of the digestive tract. This inner wall soon separates completely from the outer in the middle line, and there becomes two-layered, thus developing both entoderm and splanchnopleure. These lateral bands of entoderm now unite with the middle one and soon enclose the whole yolk and the "yolk cells" which latter later degenerate and are absorbed.—*J. S. K.*

MICROSCOPY.[1]

VITAL INFUSION OF NERVES WITH METHYL-BLUE.—Prof. Ehrlich's[2] experiments in staining nerves with *methyl-blue*, mark the second great advance in staining *living* tissues. The discovery of a veritable nerve-stain, that acts *intra vitam*, has already led to the settlement of many disputed points, and promises to furnish a means for deciding the problem of nerve-endings. The well-known color-reaction induced in newly formed bone-substance as the result of madder-feeding, is outshone by this wonderful reaction between methyl-blue and the axis-cylinder.

According to Ehrlich two conditions are necessary in order to get the methyl-blue reaction: These are (1) *saturation with oxygen* and (2) *alkaline reaction.* The first condition can be secured only by free exposure of the object to the air. If a cover-slip is used, the air is thus excluded, and the stain rapidly disappears.

As to the second condition, it is known that nerves at rest have an alkaline reaction. It is necessary, therefore, to experiment on *resting* nerves. The state of rest may be forced by severing the nerves before infusion with methyl-blue, or by poisoning the animals with curare. The nerve-endings of the retina may be studied on animals that have been kept some time in the dark before infusion. The number of nerve terminations that stain under ordinary conditions is, however, so great, that a wide field for histological investigation is open to us without having recourse to the expedients just mentioned.

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[1] Edited by C. O. Whitman, Director of the Lake Laboratory, Milwaukee.

[2] P. Ehrlich. Ueber die Methylenblaureaction der lebenden Nervensubstanz. Deutsch. med. Wochenschr., 1886, No. 4.

The evanescence of the color-reaction is a serious drawback, but this difficulty has been met with some success by the use of iodine and picro-carmine as "fixing" reagents.

The method of procedure, according to Prof. C. Arnstein,[1] and his pupils, Dogiel and Smirnon, is as follows:—

1. Inject the *vena cutanea magna* of a frog with 1 cc. of a saturated solution of methyl-blue. The tongue and palate are at once colored, but the coloring substance is confined to the blood-vessels, and does not at first affect the nerves. After an hour or two the nerves supplying the taste papillæ appear blue, and at the same time the nerve-meshes of the palate are also stained. The motor nerve-ends show the stain a little later. The color-reaction only lasts a short time, often not more than five to ten minutes. It should be fixed at the moment of its greatest intensity. If iodine is used for this purpose, proceed as follows:—
2. Place the frog in a 1 per cent. aqueous solution of potassic iodide, in which metallic iodine has been dissolved to saturation, and inject the blood-vessels with the same solution, thus freeing them from the blood as far as possible.
3. Next cut out the parts needed and leave them in the iodine solution from six to twelve hours.
4. Transfer to water and leave until most of the iodine has been withdrawn. As the result of this treatment, the nerves will have a dark brown or grey color, and the surrounding tissue will be nearly colorless.
5. Mount in acidified glycerine.

Picro-carmine as a "fixing" agent, is said to give more durable preparations than iodine, but the latter gives the more intense color.

Mammals and birds die soon after injection of methyl-blue, so that a true intra vitam reaction can not be easily obtained. However, a very satisfactory reaction may be obtained, with these animals, after death from chloroform. The injection may be made from the heart or from any blood-vessel. The appearance of color in the nerves can be followed under the microscope, and when its maximum has been reached, the fixing reagent applied.

Finally, with a dilute solution of methyl-blue, preparations can be stained *directly on the slide.* The retina of fishes, birds, and mammals, can be more successfully stained this way than by injection.

Dr. Max Joseph[2] has tested Ehrlich's method on Heteropods and found that the clear intra vitam stain could not be satisfactorily fixed. He remarks that *the commercial methyl-blue is unfit for use,* and that *only the chemically pure article will give the results obtained by Ehrlich.*

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[1] Anat. Anz. ii., 1887, No. 5, p. 125, and No. 17, p. 551.

[2] Anat. Anz., June, 1888, No. 15, p. 420.

Instead of a saturated solution, Dr. Joseph recommends the strength originally employed by Ehrlich,—*one-quarter gram color in 100 grams of the physiological salt solution.*

The best stain was reached about six hours after injection in the body-cavity.

Biedermann[1] has employed nearly the same method for Crustacea and insects.

CENTRAL TERMINATION OF THE OPTIC NERVE IN VERTEBRATES.[2]—For tracing the course of nerve-fibres, the following method has been employed with great success by Prof. Bellonci.

1. The brain, or a part of it containing the nervus opticus, is placed in osmic acid (½ to 1 per cent.) for fourteen to twenty hours.
2. Sections are then made with free hand in alcohol of 70 per cent.; the sections are washed in distilled water a few minutes, and then placed in 80 per cent. alcohol three or four hours.
3. The sections are again placed in distilled water, and then transferred to the object-slide, and covered with a cover-slip.
4. A few drops of ammonia are then allowed to mix with the water under the cover-slip. This reagent makes the brain transparent as glass, with exception of the nerve-fibres, which remain black, and which are brought out with such distinctness that their course is easily followed.

The sections are of course thick, but this is an advantage in tracing the winding course of the fibres.

Sections cut in celloidin with the microtome can be treated in same manner, but the action of the ammonia is much slower, requiring several days.

DOUBLE-STAINING OF THE CENTRAL NERVOUS SYSTEM.[3]—

1. Of the various fluids used for hardening, a *3 to 4 per cent. solution of bichromate of potassium* gives the best results. The object must be in a fresh condition when placed in the fluid, and the fluid must be changed often during the process of hardening.
2. The sections are best when made, without imbedding in celloidin, from pieces fastened to cork with gum and then placed in alcohol long enough to harden the gum.[4]

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[1] Sitzb. d. Kaiz. Akad. d. Wissensch., Vol. xcvi., 3 Abth. 1887.

[2] *Josef Bellonci.* Ueber die centrale Endigung des Nervus opticus bei den Vertebraten.
Zeitschr. f. wiss. Zoologie, xlvii., I., p. 4, September, 1888.

[3] *Hermann Sahli.* Ueber eine neue Doppelfärbung des centralne Nervensystems. Zeitsch. f. wiss. Mikroskopie, ii., I., p. 1, 1885.

[4] Preparations left several days in alcohol can still be used. But the sections should be laid in the bichromate of potassium for an hour, and

3. The sections are placed in water for a few minutes (not over five or ten), and then in a saturated aqueous solution of *methyl-blue* until stained deep blue.

4. They are then washed, and placed in a saturated aqueous solution of acid fuchsin for about five minutes.

5. The sections are next to be quickly washed, and placed for a *few seconds* in an alcoholic solution of caustic potash (1 per cent.), from which they are to be transferred at once to abundant water.

The color differentiation at once appears: The white matter becomes blue or violet, and the gray matter red. Bundles of fibres in longitudinal section appear to be made up partly of blue and partly of red fibres. Cross-sections show that the difference in color among the fibres is due to the presence in varying amount of two unlike substances in the medullary sheaths. These substances may be distinguished as *erythrophilous* (red) and *cyanophilous* (blue). The axis-cylinder is uniformly red, while the medullary sheaths are variegated. In some fibres the whole sheath is made up of cyanophilous matter, in others of erythrophilous matter. In the majority of the fibres, the sheath is composed of concentric layers, blue alternating with red.

In the gray matter of the spinal chord may be seen Gerlach's net-work of fine fibrils. Close examination shows that the fibril is differentiated into red axis-cylinder and blue medullary sheath.

Preparations after the above method are not permanent, but they sometimes keep for a year or more.

Such preparations show that the medullary sheath is a structure of more importance than has generally been supposed by physiologists and pathologists. The differences brought out by this process of double-staining appear to indicate a difference in function among the nerve-fibres. The division into *motor* and *sensory* fibres, as Sahli suggests, may not go to the root of the matter. The central nervous system may be built up on a much more complicated principle of division.

PROCEEDINGS OF SCIENTIFIC SOCIETIES.

KENT SCIENTIFIC INSTITUTE OF GRAND RAPIDS, MICH.—The following officers were elected to serve for the year 1888: President, E. S. Holmes; Vice-President, W. A. Greeson; Recorder, C. A. Whittemore; Corresponding Secretary, E. S. Holmes; Treas-

then washed a few minutes in water (until visible streams of the solution cease to issue from the preparation), before immersion in the staining fluid. The staining may be successful with celloidin sections, provided they are very thin. It is better, however, to remove the celloidin, and subject the sections again to the hardening fluid, preparatory to staining.